The Benign Concentric Annular Macular Dystrophy Locus Maps to 6p12.3-q16

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Purpose. To describe the clinical findings and to identify the genetic locus in a Dutch family with autosomal dominant benign concentric annular macular dystrophy (BCAMD).

Methods. All family members underwent ophthalmic examination. Linkage analysis of candidate retinal dystrophy loci and a whole genome scan were performed. Five candidate genes from the linked locus were analyzed for mutations by direct sequencing.

Results. The BCAMD phenotype is initially characterized by parafoveal hypopigmentation and good visual acuity, but progresses to a retinitis pigmentosa-like phenotype. Linkage analysis established complete segregation of the BCAMD phenotype (maximum multipoint LOD score, 3.8) with DNA markers at chromosome 6, region p12.3-q16. Recombination events defined a critical interval spanning 30.7 cM at the long arm of chromosome 6 between markers *D6S269* and *D6S300*. This interval encompasses several retinal dystrophy loci, including the *ELOVL4* gene, mutated in autosomal dominant Stargardt disease, and the *RIM1* gene, mutated in autosomal dominant cone-rod dystrophy, as well as the retinally expressed *GABRR1* and *-2* genes. Mutation screening of these four genes revealed no mutations. Sequence analysis of the interphotoreceptor matrix proteoglycan 1 gene *IMPG1*, also residing in the BCAMD locus, revealed a single base-pair change (T→C) of nucleotide 1866 in exon 13, resulting in a Leu579Pro amino acid substitution. This mutation was absent in 190 control individuals.

Conclusions. Significant linkage was found for the BCAMD defect with chromosomal 6, region p12.3-q16. A Leu579Pro mutation in the *IMPG1* gene may play a causal role. (*Invest Ophthalmol Vis Sci.* 2004;45:30-35) DOI:10.1167/iovs.03-0392

Benign concentric annular macular dystrophy (BCAMD; OMIM 153870; Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is initially characterized by parafoveal hypopigmentation together with relatively good visual acuity.[1] Patients do not have a history of chloroquine ingestion nor do they show symptoms typical for cone dystrophy, such as photophobia and severely defective color vision.[2,3] Several other patients have been reported with clinical features similar to this initial stage of BCAMD, including those in a three-generation family,[4] and six isolated patients.[5-7] A 10-year follow-up of the Dutch family revealed a more pronounced involvement of the peripheral retina together with increased photoreceptor cell dysfunction, as found in retinitis pigmentosa (RP).[8] Currently, 12 genetic loci are associated with nonsyndromic autosomal dominant RP and 18 loci are associated with autosomal dominant dystrophies of the retina and choroid, with primary involvement of the macular area (http://www.sph.uth.tmc.edu/retnet).[9] In this study, we describe the progression of the BCAMD clinical features during the past 15 years, and we report the results of a genome-wide search undertaken to find the gene responsible for BCAMD in this family. We detected linkage with chromosome 6, region p12.3-q16, and subsequently analyzed five candidate genes residing in this chromosomal region.

Methods

Clinical Studies

The study protocol adhered to the tenets of the Declaration of Helsinki. Fifteen relatives from the original Dutch family with BCAMD were included in this study. Informed consent was obtained from all participants. The medical histories were obtained from 5 unaffected and 10 affected individuals. Subsequent ophthalmic examinations included best corrected Snellen visual acuity determination, fundus examination, and fluorescein angiography. An electroretinogram (ERG) and an electrooculogram (EOG) were obtained according to the International Society for Clinical Electrophysiology of Vision protocol (http://www.iscev.org). Visual field examination was performed monocularly with a Goldmann perimeter. In addition, several patients underwent color vision and dark-adaptation testing.

Molecular Genetic Studies

Linkage Analysis. Blood samples of 19 individuals from three generations were collected and DNA was isolated as described elsewhere.[9] Microsatellite markers were chosen from the Généthon database (www.genethon.fr; provided in the public domain by the French Association against Myopathies, Evry, France)[10] to investigate the candidate loci for autosomal dominant RP and autosomal dominant macular dystrophy. Simultaneously, a whole genome scan was performed with highly polymorphic DNA markers, spaced between 10 and 15 cM, which were derived from Généthon (available on request from the authors).[10] DNA samples were subjected to polymerase chain reaction (PCR) amplification with a standard cycling profile of 30 cycles at 94°C, 55°C, and 72°C, for 30 seconds at each step. DNA markers were

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TABLE 1. Clinical Details of the BCAMD Family

No.	Year, age at Examination	VOD	VOS	ERG (μV)				EOG		DA Elevation (10^{log})
				Sco OD	Sco OS	Pho OD	Pho OS	OD	OS	
II-2	'74 44	20/25	20/25	SN	SN	SN	SN	1.87	1.91	1.0
	'84 54	20/40	20/25	NR	NR	SN	SN	1.11	0.97	1.0
II-4	'84 50	20/25	20/20	SN	SN	SN	SN	1.12	1.00	0.5
	'99 65	20/30	20/25	NR	NR	NR	NR	0.98	0.96	1.5
II-7	'84 45	20/25	20/25	SN	SN	SN	SN	1.50	1.70	0.5
	'99 60	20/25	20/25	NR	NR	SN	SN	1.40	1.30	1.0
II-9	'99 47	20/25	20/25	NR	NR	N	N	0.98	1.04	0.5
III-2	'74 18	20/60	20/25	SN	SN	SN	SN	1.00	1.00	NP
	'84 28	20/60	20/40	NR	NR	NR	NR	1.00	1.00	1.5
	'99 43	20/60	20/40	NR	NR	NR	NR	1.09	0.97	3.0
III-3	'74 16	20/20	20/20	N	N	SN	SN	2.14	2.36	N
	'84 26	20/15	20/15	SN	SN	SN	SN	2.00	1.75	N
	'99 41	20/15	20/40	NR	NR	N	N	1.65	1.38	NP
III-5	'84 24	20/25	20/25	SN	SN	SN	SN	1.22	1.27	0.5
	'99 40	20/20	20/15	NR	NR	NR	NR	0.89	0.77	N
III-6	'99 33	20/20	20/20	NR	NR	SN	SN	1.00	1.00	1.0
IV-1	'99 17	20/15	20/15	SN	SN	N	N	1.64	1.75	N
IV-3	'02 22	20/25	20/25	SN-NR	SN-NR	SN	SN	1.00	1.00	NP

VOD, visual acuity right eye; VOS, visual acuity left eye; Sco, scotopic; Pho, photopic; EOG, electrooculography; DA, dark adaptation; N, normal; SN, subnormal; NR, nonrecordable; NP, not performed.

labeled by the incorporation of $\alpha[^{32}P]$-dCTP, and the products were separated by electrophoresis on a 6.6% denaturing polyacrylamide gel. We calculated two-point LOD scores using the subroutine MLINK of the LINKAGE program (version 5.1; http://www.hgmp.mrc.ac.uk/; provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge, UK).[11–13] Multipoint analysis was performed by means of four-point linkage analysis (FASTLINK version 2.30; http://softlib.cs.rice.edu; provided in the public domain by Rice University, Houston, Texas).[14,15] A gene frequency of 0.0001 and a penetrance of 95% were assumed for the disorder.

Mutation Screening. For sequence analysis of the *IMPG1* gene, exons 1 to 17 were amplified using oligonucleotides as described.[16] Twenty-five nanograms of genomic DNA was denaturated for 5 minutes at 95°C and subjected to 35 cycles of PCR, with the following conditions: denaturation for 30 seconds at 94°C, annealing for 30 seconds at temperatures varying from 49°C to 58°C, and extension for 30 seconds at 72°C. Finally, an extension was performed for 5 minutes at 72°C. To screen for the presence of the mutation in control individuals and to analyze the segregation of the mutation in the pedigree, an amplification refractory mutation system (ARMS) PCR for *IMPG1* 1866T→C was developed.[17] PCRs were performed using the same reverse primer of exon 13 of the *IMPG1* gene,[16] and in one PCR the wild-type specific forward primer 5′-TGGTAGTGTTCTTCAGTCT-3′ and in the other PCR the mutant-specific forward primer 5′-TGGTAGTGTTCTTCAGTCC-3′. The PCR conditions were the same as mentioned earlier, with annealing temperatures of 56°C and 59°C, respectively. The PCR products were analyzed on a 1.5% agarose gel. Sequence analysis of the *ELOVL4* and *RIM1* genes was performed using primers and conditions as described before.[18,19] The *GABRR1* and *-2* genes were screened for mutations by direct sequencing of exons and exon-intron boundaries with PCR primers and conditions available from the authors on request.

RESULTS

Clinical Findings

The clinical features are shown in Table 1. The genetic defect is inherited in an autosomal dominant manner (Fig. 1). Visual acuity was normal at the age of onset, but decreased gradually after the fourth to fifth decade to 20/40, except for individual III-2. Her visual acuity was 20/60 in the right eye, due to amblyopia. She also underwent retinal detachment surgery in both eyes at the age of 15. Six patients reported night blindness. In the affected individuals, fundus disorders ranged from macular pigment alterations to bull's eye maculopathy and from minimal peripheral pigment disturbance to a pigmentary dystrophy with bone corpuscles. In late stages of the disease, waxy optic atrophy and attenuated arterioles were found (Fig. 2). Electroretinographic testing revealed photoreceptor dysfunction, even at a young age (individuals III-2, III-6, IV-1, IV-3 at the ages of 28, 33, 17, and 22 years, respectively) with a slight predominance of rod dysfunction above cone dysfunction. EOG was almost invariably subnormal (1.5–1.8) or abnormal (<1.5) and deteriorated with age. Only in individuals III-3 at the ages of 16 and 26 and II-2 at the age of 44 was the EOG normal (>1.8) or subnormal. The dark-adaptation curves decreased to a normal or moderately elevated level, except for patient III-2, in whom the curve was elevated as the result of previous retinal detachments. Visual fields varied from normal to a ringlike zone of decreased sensitivity surrounding a small central island of mildly decreased sensitivity, to constricted visual fields in a later stage. The color vision examination revealed a congenital deuteranomaly in the three male patients and an acquired blue-yellow defect in all affected individuals, except III-5 and IV-1. Individual IV-3 was not tested.

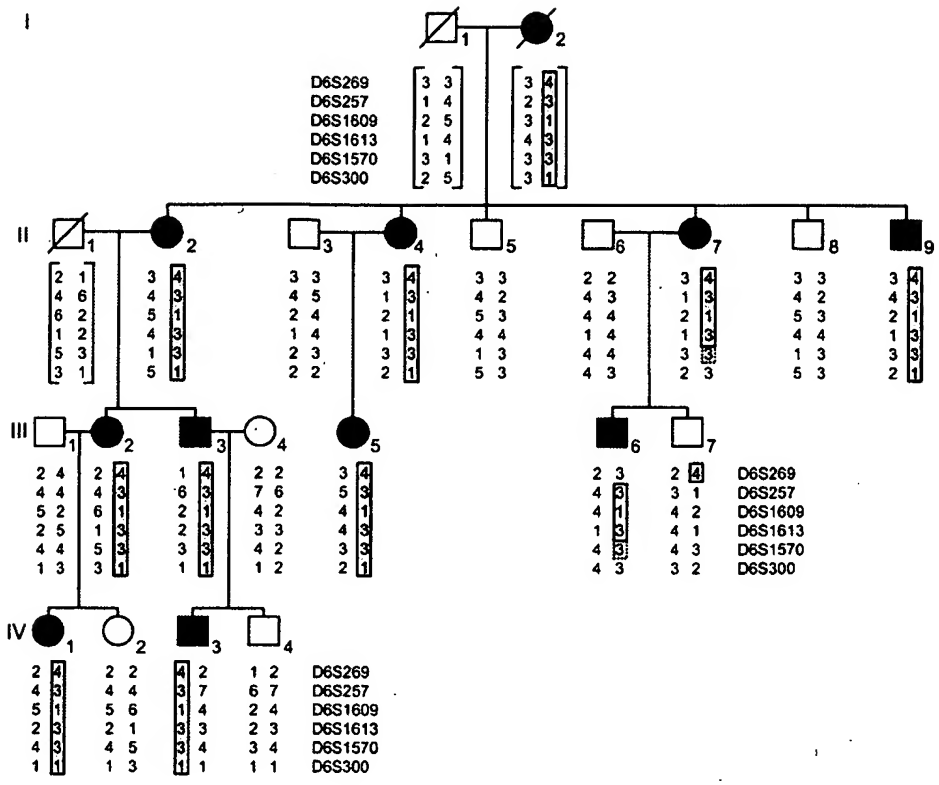

FIGURE 1. Pedigree and linkage analysis results of the BCAMD family. The 6p (between *D6S269* and *D6S257*) and 6q (between *D6S1570* and *D6S300*) boundaries of the critical interval at 6p12.3-6q16 are determined by recombination events observed in individuals II-7 and III-6. Note that marker *D6S1570* is noninformative based on the deduced haplotypes in individuals I-1 and I-2. The order of the markers is derived from the human genome project working draft (freeze Nov 2002; at http://genome.cse.ucsc.edu/) and Généthon.[10] Marker alleles between *brackets* are deduced. At risk haplotypes are boxed. *Stippled boxes*: noninformative markers in one of the parental haplotypes.

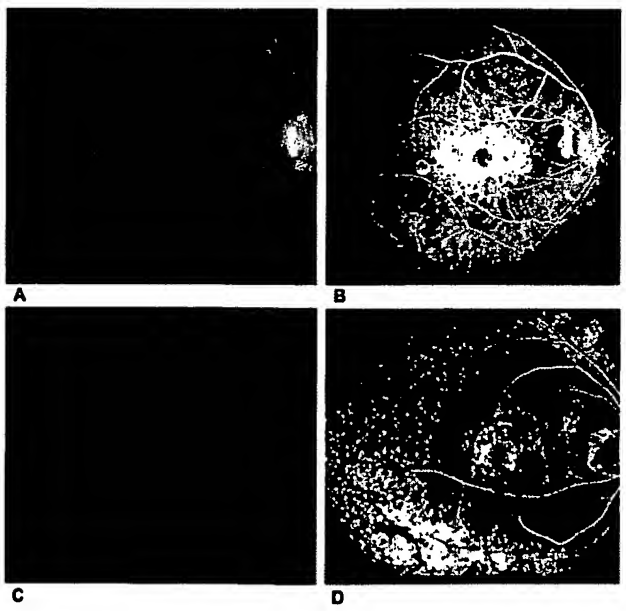

FIGURE 2. (A) Fundus photograph of patient II-9, age 47 years: Typical bull's-eye maculopathy and slightly attenuated arterioles; (B) fluorescein angiogram of patient II-9, age 47 years: ringlike macular hyperfluorescence, surrounded by mid-peripheral diffuse granular hyperfluorescence. (C) Fundus photograph of patient II-4, age 65 years: bull's-eye maculopathy, attenuated arterioles, bone corpuscle pigmentations, peripheral chorioretinal atrophy. (D) Fluorescein angiogram of patient II-4, age 65 years: irregular macular fluorescence, areas of choriocapillaris atrophy. In the midperiphery diffuse granular hyperfluorescence and bone corpuscle pigmentations.

Molecular Genetic Findings

Linkage Analysis. Using linkage analysis the following candidate loci for X-linked and autosomal dominant RP were analyzed: Xp21.1 (RP3), 1q21.1 (RP18), 3q21-q24 (RP4), 6p21.2-cen (RP7), 7p15.1-p13 (RP9), 7q31.3 (RP10), 17p13.1 (RP13), 17q22 (RP17), 19q13.3 (*CRX*), and 19q13.4 (RP11). Not tested were the loci 8q11-q13 (RP1), 14q11.2 (RP27), and 17q25 (*FSCN2*). Likewise, the following loci for X-linked and autosomal dominant macular dystrophy were tested: Xp21.1 (COD1), Xq27 (COD2), 1q25-q31 (ARMD1), 2p16-p21 (DHRD), 4p (STGD4), 6p21.1 (COD3), 6p21.2-cen (RDS), 6q13-q14 (CORD7), 6q14 (STGD3), 6q14-q16.2 (PBCRA, MCDR1), 6q25-q26 (RCD1), 7p21-p15 (CYMD), 11q13 (VMD2), 17p13 (CACD), 17p13.1 (CORD6), 17p13-p12 (CORD5), 17q (CORD4), 17q11.2 (*UNC119*), 19q13.3 (CORD2), and 22q12.1-q13.2 (SFD). All loci except 6q14 (STGD3) and 6q13-q14 (CORD7) were excluded. The genetic defect cosegregated with markers from 6p12.3-q16. In the genome scan, except for the 6p12.3-q16 region, multiple recombinations were found in affected family members with all markers analyzed. Two-point LOD scores between the relevant markers from 6q12.3-q16 and the disease phenotype are given in Table 2. A maximum LOD score of 3.81 at $\theta = 0.0$ was obtained with markers *D6S1609* and *D6S1613*. Multipoint analysis with the markers *D6S257*, *D6S1609*, and *D6S1570* resulted in a maximum LOD score of 3.81 in the whole region between *D6S257* and *D6S1570*. The analysis of the recombination events in individuals II-7 and III-6 revealed a critical region on chromosome 6 between markers *D6S269* and *D6S300*, which flank a 30.7-cM interval (Fig. 1). In this region, the loci for autosomal dominant Stargardt disease (STGD3),[19] dominant drusen (DD),[20] and autosomal dominant cone rod dystrophy (CORD7)[21] have been positioned, as well as two loci for autosomal recessive disorders—that is *RP25* and Leber congenital amaurosis (*LCA5*)[22,23] (Fig. 3).

TABLE 2. Two-Point LOD Score Data of the BCAMD Family for Chromosome 6 Microsatellite Markers

Marker	Recombination Fraction							Z_{max}	θ_{max}
	0.00	0.01	0.05	0.1	0.2	0.3	0.4		
D6S269	−3.99	0.31	0.96	1.13	1.04	0.71	0.32	1.13	0.1
D6S257	3.51	3.44	3.18	2.83	2.09	1.27	0.45	3.51	0.0
D6S1609	3.81	3.74	3.48	3.13	2.38	1.54	0.63	3.81	0.0
D6S1613	3.81	3.74	3.48	3.13	2.38	1.54	0.63	3.81	0.0
D6S1570	1.05	1.02	0.93	0.81	0.60	0.40	0.19	1.05	0.0
D6S300	−4.66	0.90	1.40	1.45	1.20	0.77	0.29	1.45	0.1

Mutation Analysis. Candidate genes located in the BCAMD locus at chromosome 6 include *ELOVL4*, *GABRR-1*, *GABRR-2*, *RIM1*, and *IMPG1* (Fig. 3). *ELOVL4* is involved in the biosynthesis of very long chain fatty acids and mutated in patients with autosomal dominant STGD3.[19,26] The *GABRR1* and *-2* genes encode the γ-aminobutyric acid (GABA) rho-1 and rho-2 subunits of the GABA-C receptor, which is the effector of lateral inhibition in the retina.[27,28] The Rab3A-interacting molecule (*RIM1*) gene, which is expressed in brain and photoreceptors, is involved in photoreceptor synaptic vesicle priming and exocytosis and mutated in a family with autosomal dominant CORD.[18] We analyzed the 6 protein coding exons of *ELOVL4*, the 9 protein coding exons of *GABRR1*, the 9 protein coding exons of *GABRR2*, and the 35 protein coding exons of *RIM1* in two patients with BCAMD. No sequence alterations were detected in the open reading frames of these four genes in the BCAMD-affected family. *IMPG1* encodes the interphotoreceptor matrix proteoglycan-1 protein, a major retina-specific glycoprotein of the interphotoreceptor matrix (IPM).[29,30] Sequence analysis of the 17 exons of *IMPG1* in the BCAMD family revealed a T to C change at nucleotide (nt) 1866 in exon 13 resulting in a Leu579Pro substitution (Fig. 4). ARMS analysis showed that this mutation segregated with the BCAMD phenotype (Fig. 5) and was not found in 190 control individuals (data not shown). Furthermore, a previously described H518D polymorphism in exon 13[16] and a 2162G→A alteration in exon 15, resulting in an R711H substitution, were found. The latter DNA variant was also found in 6 of 20 DNAs of control indi-

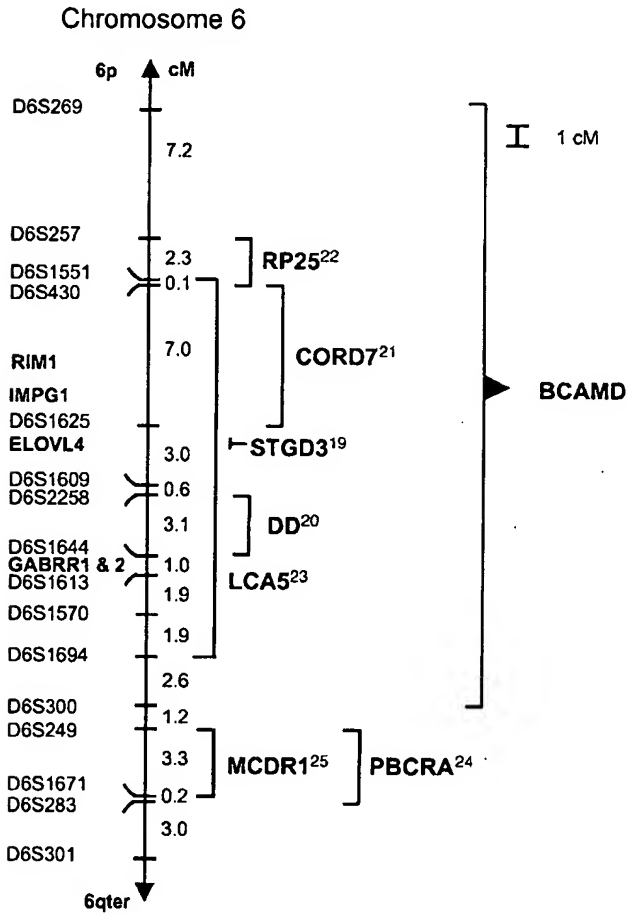

FIGURE 3. A genetic and morbidity map of 6p12.3-6q16. The linkage intervals for families with Leber congenital amaurosis (LCA5), RP25, progressive bifocal chorioretinal atrophy (PBCRA),[24] North Carolina macular dystrophy (MCDR1),[25] CORD7, STGD3, DD, and the BCAMD-affected family are depicted, as well as the localizations of the *RIM1*, *ELOVL4*, *GABBR1* and *-2*, and *IMPG1* genes. The localization of the markers and the genetic distances (in centimorgans) are derived from the human genome project working draft (freeze Nov 2002; at http://genome.cse.ucsc.edu/) and Généthon.[10]

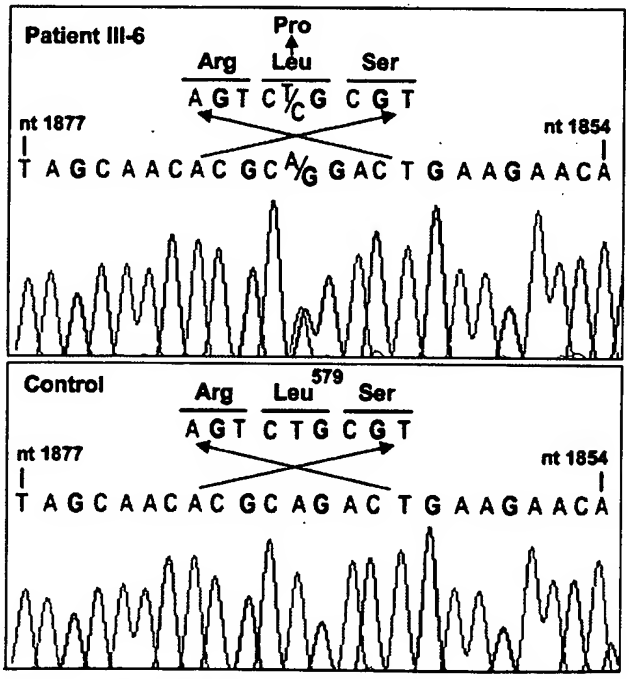

FIGURE 4. DNA sequence analysis of part of exon 13 of *IMPG1*. *Top*: Antisense sequence of patient III-6, carrying a heterozygous T→C transition at nt 1866, resulting in an amino acid change Leu579Pro. *Bottom*: Antisense sequence of a control individual is shown for comparison. *Above* these sequences part of the complementary sense sequence is depicted.

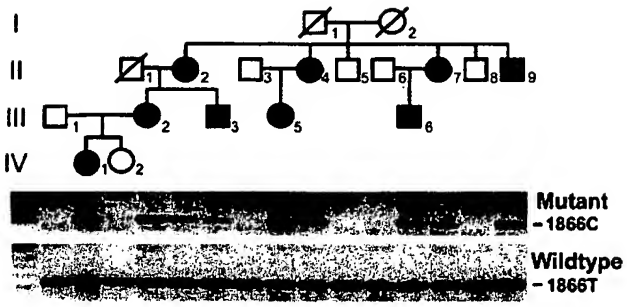

FIGURE 5. Amplification refractory mutation system assay showing the inheritance of the Leu579Pro mutation in a large part of the family (III-4, III-7, IV-3, and IV-4 were not tested and therefore are not depicted in the figure). *Top*: PCR product of the mutant primer pair. *Bottom*: PCR product of the wild-type primer pair. At the *left*, a 100-bp ladder is present. Both PCR products migrate between 500- and 600-bp fragments.

viduals (data not shown) rendering it a frequent polymorphism.

DISCUSSION

We performed a 15-year follow-up of a BCAMD-affected family. Affected members present with a ringlike depigmentation around the fovea, associated with relatively good visual acuity in the third decade, which slowly decreases in the fourth to fifth decade. The progression of the phenotype is indicative of RP with a bull's-eye configuration, because of the more pronounced involvement of the peripheral retina and the increased photoreceptor dysfunction, with slight predominance of rod dysfunction even at an early stage. It is hard to differentiate BCAMD from cone–rod dystrophy, but progressive central acuity loss and photoaversion are not typical findings at any stage of disease, whereas night blindness is found at young age in some patients. Only one three-generation family[4] and six sporadic patients with a clinical entity similar to BCAMD[5,6] have been described earlier. However the follow-up of these patients is unknown, which complicates comparison of these cases with patients from our family.

By performing extensive linkage analysis, we observed full cosegregation of the BCAMD phenotype with DNA markers of 6p12.3-q16 with maximum two-point (3.8) and multipoint LOD scores (3.8). Mutation analysis of the *ELOVL4*, *GABRR1* and *-2*, *RIM1*, and *IMPG1* genes on chromosome 6 revealed a Leu579Pro mutation in *IMPG1*. *IMPG1* and its homologue *IMPG2* encode the chondroitin sulfate proteoglycan core proteins, interphotoreceptor matrix (IPM) 150/SPACR and IPM 200/SPACRCAN, respectively. *IMPG1* is expressed in the retina;[31] *IMPG2* has been detected around rod and cone photoreceptors and in pinealocytes.[31,32] Chondroitin-6-sulfate-rich glycosaminoglycans have been identified around cone photoreceptor cells and have been named "cone matrix sheets."[33,34] These molecules appear to play a crucial role in the physical attachment of the neural retina to the RPE.[35–41] The human *IMPG1* and *IMPG2* proteins consist of 771 and 1160 amino acids, respectively. Both contain a predicted signal peptide at the amino terminus, and they show two regions with high sequence homology (domain C1: Arg^{67}-Trp^{126} and domain C2: Leu^{573}-Val^{654}).[42,43] They contain one (*IMPG1*) or two (*IMPG2*) putative EGF-like domains thought to be involved in protein–protein interaction. Both contain a putative hyaluronan-binding motif and *IMPG2* contains three putative glycosaminoglycan-binding motifs.[31,44] It is thought that *IMPG1* and *IMPG2*, through their binding with hyaluronan, may serve to organize the basic macromolecular scaffold for the IPM.[31]

The mutated leucine residue at position 579 resides in the second conserved region (domain C2 Leu^{573}-Val^{654}) and is present in human IMPG1, human IMPG2, mouse Impg1, and rat Impg1.[31,44–46] According to a secondary structure prediction program (http://www.embl-heidelberg.de/predictprotein, provided in the public domain by the European Molecular Biology Laboratory, Heidelberg, Germany) the Leu579Pro mutation has no major effect. The *IMPG1* amino acid residues 568-571 are predicted to form a coiled coil. The residues 572-582 form a β-sheet, which is followed by a coiled coil (residues 583-586). The Pro-for-Leu exchange at 579 merely predicts a stronger β-sheet for residues 578-582. An LCA5 family has been tested for mutations in *IMPG1*, but no alterations were found.[23] In patients from 6q linked multigeneration families diagnosed with progressive bifocal chorioretinal atrophy and North Carolina macular dystrophy, as well as in a single patient from an autosomal dominant STGD pedigree unlinked to either of the two known STGD2 and -3 loci on the long arms of chromosomes 13 and 6, respectively, no disease-associated mutations were identified in the IMPG1 gene.[9] The *IMPG2* gene could not be implicated in 40 patients with LCA, 92 patients with RP, and 92 patients with age-related macular degeneration.[43]

In summary, the BCAMD phenotype, which progresses from a parafoveal hypopigmentation with good visual acuity into a RP-like entity, apparently is caused by a gene defect residing at chromosome 6. *IMPG1* represents an excellent functional candidate gene for BCAMD at chromosome 6 and the Leu579Pro mutation may play a causal role. However, additional patients with BCAMD and RP must be analyzed to establish causality for the *IMPG1* gene.

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